Contamination of polycyclic aromatic hydrocarbons and their implication for environmental hazard

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Abstract Studies concerning the contamination and pollution sources of polycyclic aromatic hydrocarbons (PAHs) in the Yodo River System, Japan, are summarized in this review. Mutagenicity and genotoxicity have been frequently detected in the tributary rivers of Yodo River, and novel contaminants such as phenylbenzotriazole type mutagens, nitrated PAHs, and heterocyclic amines have been suggested to be the causative agents. The contribution of nonsubstituted PAHs to the toxicity is considered to be low since only few ng/l of phenanthrene, fluoranthene, and pyrene have been found in the river waters. The application of an aryl hydrocarbon receptor (AhR) dependent bioassay in evaluating the combined toxicity caused by various pollutants in environmental samples is also introduced. Point and non-point sources of pollution discharging into the Lake Biwa-Yodo River System, including treated sewage effluents and deposited road particles, have been investigated using the AhR-dependent reporter gene assay. The results suggested the presence of newly identified AhR ligands such as anthraquinone dyes or unidentified AhR ligands in the treated sewage effluents and the road particles, respectively. Finally, toxic effects of PAHs that are mediated via AhR binding are also summarized.

Keywords Aryl hydrocarbon receptor; Lake Biwa-Yodo River System; polycyclic aromatic hydrocarbons

Introduction
Polycyclic aromatic hydrocarbons (PAHs) are a group of organic environmental contaminants that are mainly produced by natural and anthropogenic combustion processes, such as forest fire, volcano eruption, fossil fuel combustion, oil refining, or other industrial activities. PAHs are introduced into the water environment via both point and non-point source discharges, including industrial or municipal wastewater effluents, urban runoff, oil spillage, and atmospheric deposition (Manoli and Samara, 1999). Many PAHs have been reported to elicit genotoxic, mutagenic, estrogenic and anti-estrogenic activities in various biological assays (Sjögren et al., 1996; Santodonato, 1997). Moreover, the International Agency for Research on Cancer (IARC) have listed several PAHs as probable or possible human carcinogens, and U.S. EPA has classified 16 unsubstituted PAHs as priority pollutants as well.

Many analytical methods have been developed for the detection of PAHs in environmental samples. In recent years, the techniques used most frequently are gas chromatography (GC) equipped with mass spectrometry (MS) or high pressure liquid chromatography (HPLC) equipped with a fluorometric detector or a photodiode array detector (Machala et al., 2001; Ciganek et al., 2004; Koh et al., 2004; Qiao et al., 2006). The use of HPLC-MS was limited because PAHs are not easily ionized or fragmented due to their low polarity. To solve this problem, post-column derivatisation with trotylium or silver cations has been studied to produce positive ions that can be detected by HPLC coupling with electrospary ionization mass spectrometry (Moriwaki, 2000; Airiau et al., 2001; Takino et al., 2001). In addition, atmospheric pressure photoionization mass
spectrometry, a new technique that can achieve the ionization of low polarity compounds, has also been shown to be an adequate approach for the analysis of PAHs (Moriwaki et al., 2004).

Chemical analysis is capable of determining the concentrations of particular PAHs for which analytical techniques are available, but it only provides little information on the total toxicity of environmental samples containing various chemicals. In addition, it has been shown that formation of PAH derivatives such as nitrated, chlorinated or brominated pyrenes occur in surface soil or chlorine-disinfected drinking water (Murahashi et al., 2004; Hu et al., 2006). Toxicity may be misestimated without considering these derivatives or other unidentified contaminants. Therefore, numerous bioassays using wild-type and recombinant bacteria, yeast, cells have been developed to assess the combined toxic and biological effects of environmental samples. In this review, studies concerning the contamination of PAHs in the Lake Biwa-Yodo River System of Japan are summarized, and the application of an aryl hydrocarbon receptor (AhR) dependent bioassay in investigating the pollution sources of PAHs in the Lake Biwa-Yodo River System is introduced. In addition, potential toxic effects of PAHs that relate to AhR binding are also discussed.

**PAHS in the Lake Biwa–Yodo River system**
The Lake Biwa-Yodo River System (Figure 1), located in the mid-western part of Japan, is a major drinking water source for over 16 million residents (Kawanishi et al., 2004). Katsura River, Uji River, and Kizu River are three main tributary rivers of Yodo river. River waters of the Katsura River, which receive treated effluents from two sewage treatment plants, have been reported to elicit acute toxicity to *Daphnia magna* (Hosokawa et al., 1995) and show mutagenicity or genotoxicity in Ames Salmonella microsome assay (Muraoka et al., 1985; Sakamoto and Hayatsu, 1990; Sayato et al., 1993; Sakamoto et al., 1996) and umu assay (Ohe and Nukaya, 1996; Ohe, 1997), respectively. It has been indicated that possible mutagens in sewage effluents constituted the major mutagenic components in the Katsura River (Sakamoto and Hayatsu, 1990). Several unsubstituted PAHs such as anthraquinone, chrysene, benzo[a]anthracene, benzo[b]fluoranthene, or azulene and quinoline derivatives have also been found in the mutagenic HPLC fractions of Katusra River water extracts using GC-MS analysis (Sayato et al., 1993). Nagai et al. (2002) have investigated the concentrations of 17 PAHs in various treatment plants, including 6 PAHs that guideline values in drinking water have been established by WHO.

![Figure 1 The Lake Biwa-Yodo River System, Japan](https://iwaponline.com/ws/article-pdf/6/6/165/418352/165.pdf)
due to their health significance. Their results revealed that only few ng/l of phenanthrene, fluoranthene and pyrene were detected in the Katsura River, and fluoranthene was also found in the Uji River and Kizu River. The contribution of PAHs to the mutagenicity, however, was low in their studies.

Novel genotoxic or mutagenic compounds have been separated from the Nishitakase, a tributary river of Yodo River that also receives treated sewage effluents, and identified to be nitroarenes and heterocyclic amines (Ohe and Nukaya, 1996; Nukaya et al., 1997; Ohe, 1997; Oguri et al., 1998). The identified heterocyclic amines have also been detected in the river waters of Katsura River and Yodo River (Ohe et al., 1999). Sewage effluents are suggested to be the source of these contaminants, and several of them are presumed to be derivatives of dye products. Table 1 shows the novel mutagenic, genotoxic, or estrogenic contaminants detected in the Yodo River System. Table 2 shows levels of PAHs in other rivers around the world.

Investigation of PAH contamination using AhR-dependent bioassays

Aryl hydrocarbon receptor

Aryl hydrocarbon receptor is a ligand-activated transcription factor that mediates many biological and toxic effects of numerous environmental contaminants. PAHs and halogenated aromatic hydrocarbons (HAHs) represent the typical classes of AhR ligands that are planar and hydrophobic. After ligand binding, the ligand:AhR complex enters the nucleus and dimerizes with the AhR nuclear translocator (ARNT) protein. The heterodimer subsequently interact with the xenobiotic responsive element (XRE) which leads to the expression of downstream target genes (Figure 2). Responsive genes regulated by the AhR include those encoding xenobiotic-metabolizing enzymes, such as cytochrome P4501A1 (CYP1A1), CYP1A2, UDP-glucuronosyltransferase, aldehyde dehydrogenase, NAD(P)H-quinone oxidoreductase, and glutathione transferase (Sjögren et al., 1996; Denison and Nagy, 2003; Sugihara et al., 2004). Metabolization of PAHs by these enzymes may result in detoxification or formation of metabolites inducing DNA damage by forming DNA adducts.

AhR-dependent bioassays

Various AhR-dependent bioassays have been developed to detect AhR-mediated activity of environmental samples. The increased CYP1A1 expressions, which can be determined by immunoblotting or by measuring the induction of mixed function oxygenase activities such as 7-ethoxyresorufin O-deethylolation (EROD) or 7-ethoxycoumarin O-deethylolation. This can help in identifying PAH contamination levels.

Table 1 Novel contaminants identified in the Yodo River System

<table>
<thead>
<tr>
<th>Compound</th>
<th>Toxic property</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Phenylbenzotriazole type compounds (PBTA-1, PBTA-2)</td>
<td>Mutagenic, Ames test (YG 1024, S9 mix +)</td>
<td>Nukaya et al., 1997; Oguri et al., 1998</td>
</tr>
<tr>
<td>Nitroarenes (1-Nitropyrene)</td>
<td>Genotoxic, Umu assay (NM 2009)</td>
<td>Ohe and Nukaya, 1996</td>
</tr>
<tr>
<td>Heterocyclic amines (MelQx, Trp-P-1, Trp-P-2, PhIP)</td>
<td>Genotoxic, Umu assay (NM 2009, S9 mix +)</td>
<td>Ohe, 1997; Ono et al., 2000</td>
</tr>
<tr>
<td>Phytoestrogens (Genestin)</td>
<td>Estrogenic, Yeast Estrogen Screening assay</td>
<td>Kawanishi et al., 2004</td>
</tr>
</tbody>
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Compound abbreviations: PBTA-1: 2-[2-(acetylamino)-4-[bis[2-methoxyethyl]amino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benotriazole; PBTA-2: 2-[2-(acetylamino)-4-[N-(2-cyanoethyl)ethylamino]-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benotriazole; MelQx: 2-amino-3,8-dimethylimidazo[4,5-f]quinoline; Trp-P-1: 3-amino-1,4-dimethyl-5H-pyrrolo[4,3-b]indole; Trp-P-2: 3-amino-1-methyl-5H-pyrrolo[4,3-b]indole; PhiP: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine
AhR-mediated activity (Nakama et al., 1997; Nito et al., 2001; Hollert et al., 2002; Sundberg et al., 2005; Qiao et al., 2006; Xiao et al., 2006). The chemical activated luciferase expression (CALUX) assay has also been frequently used to evaluate dioxin groups by measuring the expression of an AhR-regulated luciferase reporter gene transfected into rat or mouse cell lines. This reporter gene assay has been utilized to assess the AhR-mediated activity of individual PAHs (Machala et al., 2001), industrial wastewater effluents (Zacharewski et al., 1995; Hurst et al., 2005), and sediment from sea, lake or river (Kannan et al., 2000; Gray et al., 2003; Koh et al., 2004). Information concerning AhR-dependent cell bioassays can also be found in other reviews (Seidal et al., 2000; Giesy et al., 2002; Denison et al., 2004).

Yeast-based reporter gene assay

The yeast-based reporter gene assay, developed by Dr. Charles Miller (1999), uses the recombinant yeast YCM3 strain that contains a human AhR and ARNT expression

![Figure 2](https://iwaponline.com/ws/article-pdf/6/6/165/418352/165.pdf)

**Figure 2** Mechanism of activation of gene expressions by the AhR in cells
construct and a pTXRE5-Z reporter plasmid responsive to the ligand-induced AhR/ARNT complex (AHRC). A lacZ expression vector is inserted into the pTXRE5-Z reporter plasmid, and β-galactosidase activity is measured to assess the activation of AHRC signaling. This yeast bioassay has been used to evaluate the AhR binding affinity of emerging environmental contaminants, such as novel mutagens occurred in river water or sediment (Takamura-Enya et al., 2002; Murahashi et al., 2004), halogenated derivatives of aza-PAHs or pyrene (Saeki et al., 2003; Hu et al., 2006), and several new antifouling compounds (Noguerol et al., 2006). Indirubin, a potential physiological AhR ligand, has been isolated from human urine by using this bioassay in combination with instrumental separation and identification (Adachi et al., 2001). The following introduce the application of this bioassay in investigating the AhR-mediated activity of point and non-point sources discharged into the Yodo River System.

Applications in investigating PAH contamination in the Yodo River System

Point sources - treated sewage effluents. Treated sewage effluents discharged into the Yodo River System have been investigated for the AhR-mediated activity using the yeast bioassay (Chou et al., 2006). The concentrated extract of sewage effluents showed AhR-mediated activity, and several hydrophobic HPLC fractions elicited higher activity after HPLC separation. Potential AhR ligands isolated from these HPLC fractions were subjected to chemical identification, and several industrial anthraquinone dyes, including Rhodamine B base and two possible disperse dyes, were identified using HPLC-MS/MS. In this study, however, solid phase extraction of treated sewage effluents was undertaken after filtration, thus only dissolved PAHs remained in the water samples. The contributions of dissolved PAHs to the AhR-mediated activity of the effluents was suggested to be low, since significant activity was not detected in the fractions corresponding to the retention times of PAHs activating the AhR, such as benzo[k]fluoranthene, benzo[a]pyrene, or chrysene (Chou et al., 2006; unpublished data).

Non-point sources - deposited road particles. Urban runoff, a major pollution source of PAHs in the water environment, contains solids deposited on impervious surfaces, such as particles of tyres and asphalt, particulate vehicle exhaust, or spilt oil. AhR-mediated activity of road dust samples collected at the surrounding of Lake Biwa has been investigated to assess the contamination of PAHs, HAHs, or other xenobiotic AhR ligands that may occur in the runoff flowing into the lake (Matsui et al., 2002). Road dust samples were divided into 8 portions by particle size, and the smallest screened portion (particle size <0.02 mm) elicited the strongest activity in the yeast bioassay. Particles larger than 0.25 mm, which constituted 72% of the weight of road dust samples, only accounted for 38% of the AhR-mediated activity. It was also found that the highest activity was detected in the HPLC fractions corresponding to the retention time of benzo[a]pyrene, but the contribution of benzo[a]pyrene to the activity was less than 1%.

The deposited road particles (DRPs) have also been sampled from 13 heavy traffic roadways in an urban area located in the southern part of Lake Biwa, and examined for PAH concentrations and AhR-mediated activity in seven different particle size fractions (Lee et al., 2005). The most dominant PAHs detected in the fractions of DRPs were pyrene, fluoranthene, and benzo[ghi]perylenes. The mean compositions of pyrene, fluoranthene, benzo[ghi]perylenes, and phenanthrene to the concentrations of total PAHs in different size fractions were 19–21%, 14–16%, 7–13%, and 9–12%, respectively. All fractions elicited AhR ligand activity, and the smallest size fraction showed the highest activity, which was 5 times more potent than that of the largest size fraction. Benzo[k]fluoranthene, chrysene, benzo[b]fluoranthene, and benzo[a]pyrene showed high
AhR binding affinity in the yeast bioassay, but their contributions to the activity observed in different size fractions were relatively low, ranging from 1.43–4.11%, 1.63–3.53%, 0.63–1.69%, and 0.31–1.42%, respectively. These results indicated that unidentified AhR ligands in DRPs might enter the Lake Biwa via urban runoff.

**AhR-mediated toxic or biological effects of PAHS**

Several excellent reviews have discussed the role of AhR in modulating the induction of gene expression and mediating toxic and biological responses effects of AhR ligands (Denison and Heath-Pagliuso, 1998; Fujii-Kuriyama and Mimura, 2003; Mandal, 2005; Pocar et al., 2005; Bock and Köhle, 2006). AhR-dependent responses are suggested to be species-specific, tissue-specific, and ligand-specific. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the most potent AhR ligand, elicits various specific toxic effects such as chloracne, carcinogenesis, teratogenesis, wasting syndrome, thymic atrophy, immune dysfunction, estrogenic or antiestrogenic activities, and hepatic damage. It is believed that these toxic effects relate to the continuous and altered gene expression resulting from the high AhR binding affinity and strong resistance to metabolic degradation of TCDD (Denison and Heath-Pagliuso, 1998; Pocar et al., 2005).

Inouye et al. (2002) reported that TCDD was not metabolized by any of the 12 forms of human CYP-dependent monooxygenases, however, many PAHs are transient ligands that may be detoxified or metabolically activated by the AhR-regulated CYP enzymes. Indirubin, a potential physiological ligand of the AhR that exhibits no AhR-mediated toxicity, has been shown to be a good substrate for the human CYP1A1 (Adachi et al., 2004). Benzo[a]pyrene is also easily metabolized by the CYP enzymes, but it is converted into highly reactive electrophilic metabolites, such as benzo[a]pyrene-7,8-diol-9,10 epoxide (BPDE), that bind covalently to nucleic acids and forms DNA adducts (Stansbury et al., 1994). Antiestrogenic effects caused by PAHs have also been suggested to relate to the induction of CYP enzymes, since these enzymes are able to catalyze the metabolism of steroids, including the hydroxylation of estradiol (Santodonato, 1997). In contrast, estrogenic effects of 3-methylcholanthrene has been shown be resulted from a direct interaction between the AhR/ARNT heterodimer and unliganded estrogen receptor, as well as the formation of functional unit bound to estrogen response elements that activate transcription (Ohtake et al., 2003).

The AhR-regulated induction of CYP enzymes is believed to be causative in mediating PAH toxicity and carcinogenicity. However, it has been reported that Fundulus heteroclitus embryo deformed with increased severity and frequency when exposed to PAHs coexisted with α-naphthoflavone, a CYP1A1 inhibitor, indicating the generation of more embryotoxic intermediates by CYP1A1 inhibition (Wassenberg and Di Giolio, 2004). Thus, these enzymes are suggested to be generally more protective than destructive in intact animals, and play an important role in the detoxification of PAHs (Nebert et al., 2004).

**Conclusions**

Identification of novel causative contaminants is important to avoid local specific hazardous pollution. The combination of the AhR-dependent yeast bioassay with instrumental analysis was very successful in screening and identifying novel xenobiotic AhR ligands in environmental samples. PAH contamination in the Lake Biwa-Yodo River System and the AhR-mediated toxic effects are summarized in this review. When we evaluate the river water quality as drinking water source, it has been revealed that concentrations of nonsubstituted PAHs in the river waters are generally low and their contributions to the toxicity might not be significant. However, when we evaluate the river water quality considering food chain accumulation, it raises concerns since the Yodo River is one of the major sources discharging...
contaminants into the Osaka Bay, where inshore fisheries provide significant protein sources to local consumers. The risk analysis of PAHs with other hazardous chemicals in the water environment considering food chain effects is a remaining question.

References


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